

1/7

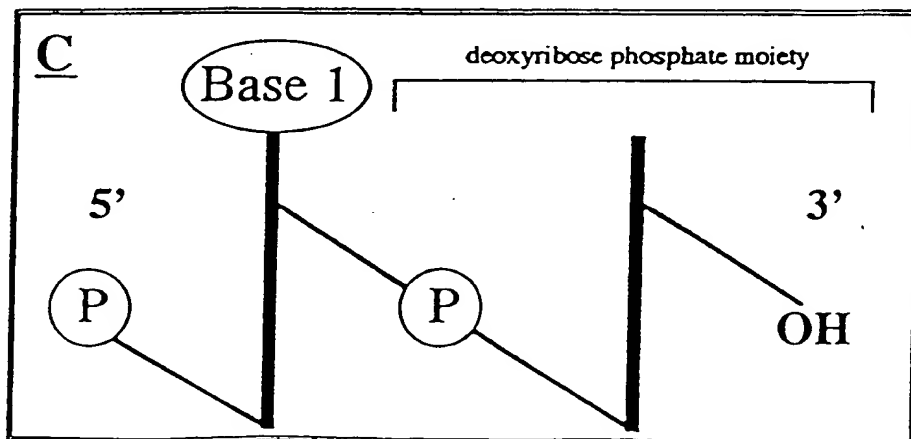
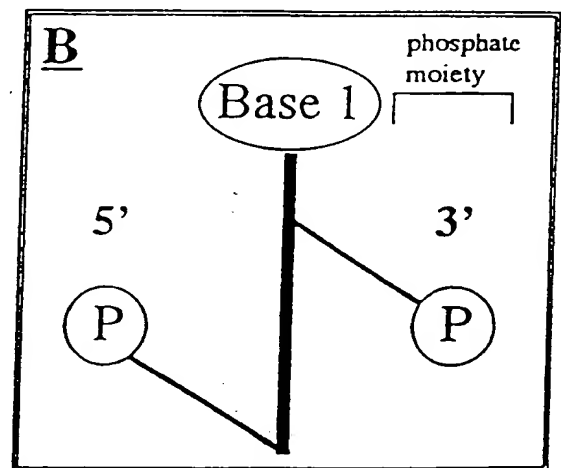
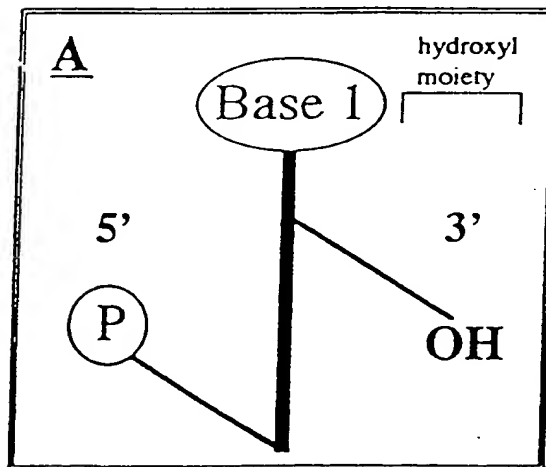
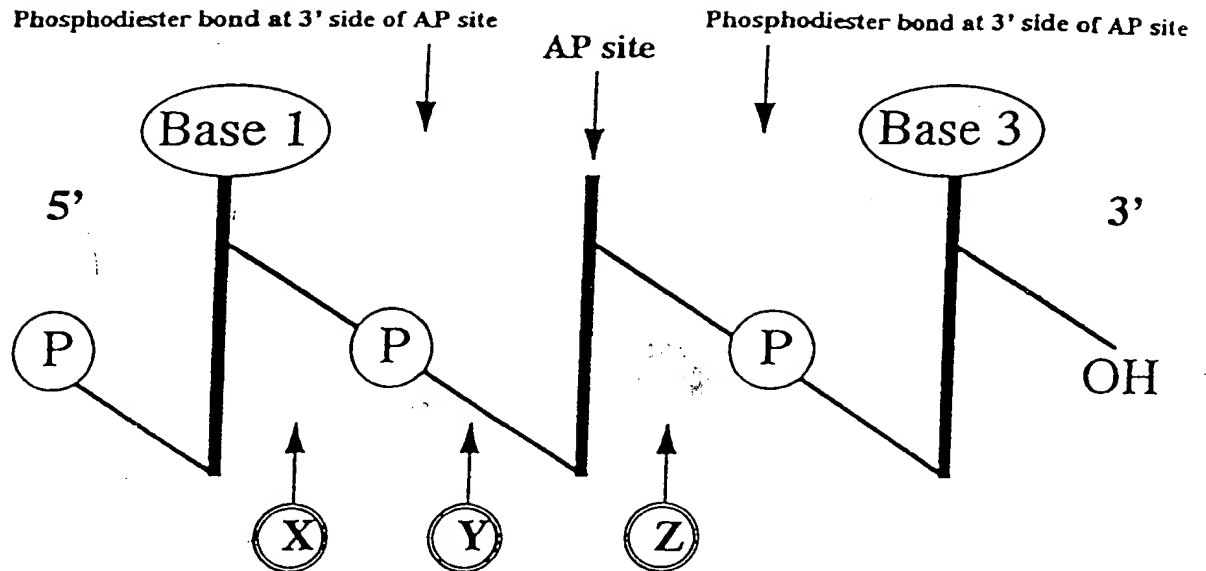
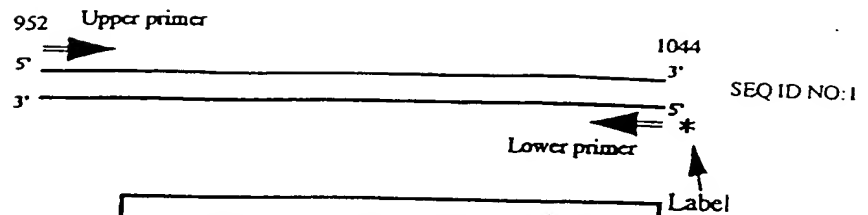
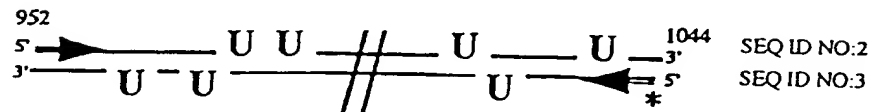


FIG 1

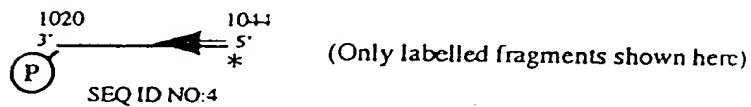
2/7



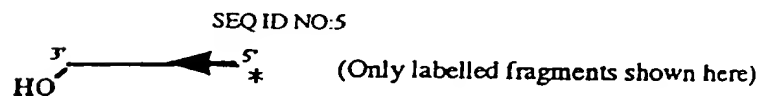
Amplification of target nucleic acid in the presence of dGTP, dATP, dUTP and dCTP and <sup>32</sup>P labelled lower primer.



- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.  
- Treatment with UDG.  
- Treatment with NaOH at 95°C



Removal of 3' phosphate by treatment with T4 PNK



Linear amplification of DNA (952 to 1044) using labelled upstream fragment followed by analysis on denaturing polyacrylamide gel, followed by autoradiography.

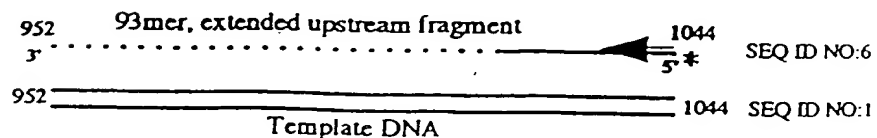
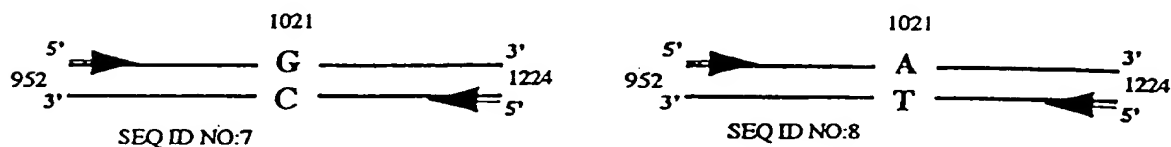


FIG 2

000207" 66282960

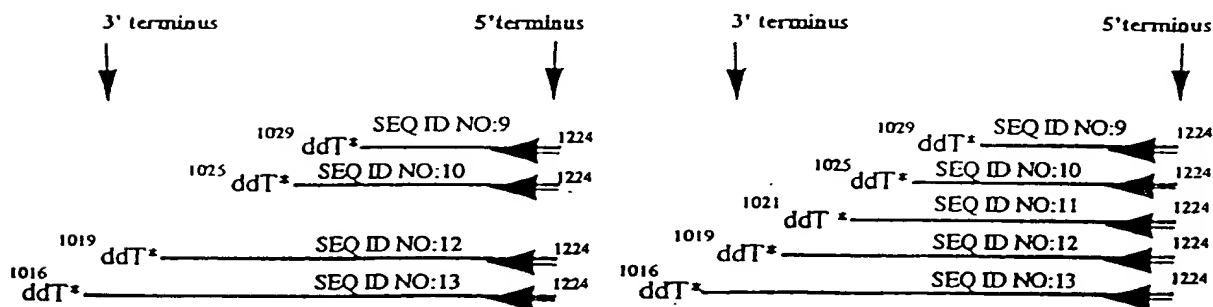
3/7

- Amplification of normal and mutant target nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.



- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95°C
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of  $^{33}\text{P}$ -labelled ddTTP\* and unlabelled ddGTP, ddATP and ddCTP.



Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.

Detection of extended labelled fragments by PAGE and autoradiography

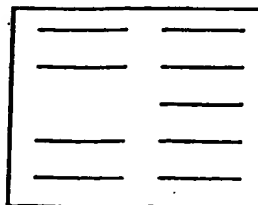
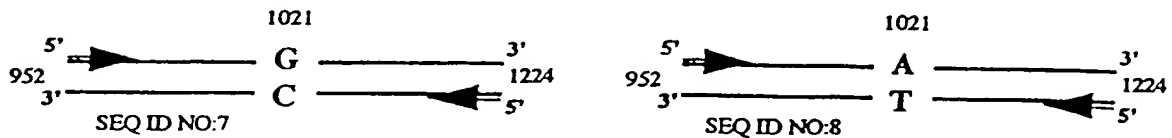


FIG 3

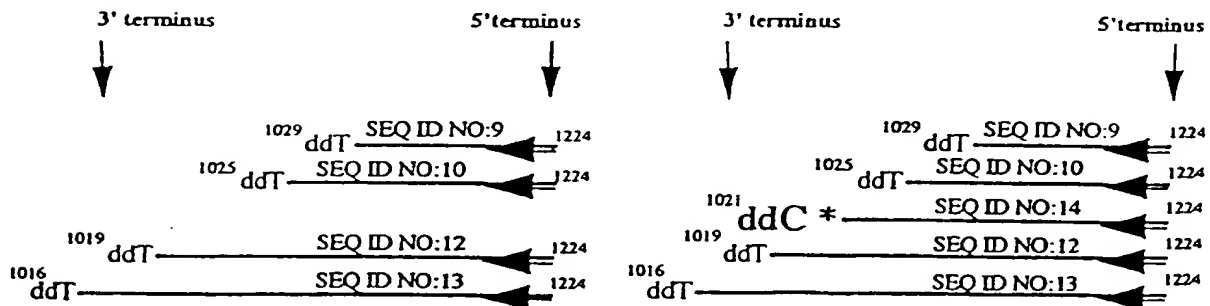
4/7

- Amplification of normal and mutant target nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.

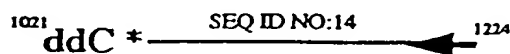


- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95°C
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of <sup>33</sup>P-labelled ddCTP\* and unlabelled ddGTP, ddATP and ddTTP.



Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.



Detection of extended labelled fragments by PAGE and autoradiography

FIG 4

000201" 68484960

5/7

6390      Upper primer      Mutation site      SEQ ID NO:15      6443

5' AACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3'

3' TTGAACACCATCAACCTCGACCACCGGATCCGTTCTCACGGAACTGCTATGTCG 5'

T      Lower primer

Amplification of target nucleic acid in the  
presence of dGTP, dATP, dUTP and dCTP.

SEQ ID NO:16

Amplified normal allele

5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'

3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

SEQ ID NO:17

SEQ ID NO:18

Amplified mutant allele

5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'

3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

SEQ ID NO:19

- Treatment with Exonuclease I  
and Shrimp Alkaline Phosphatase.  
- Treatment with UDG.  
- Treatment with Endo IV.

SEQ ID NO:20

Normal upstream fragment

3' CGACCACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

SEQ ID NO:21

Mutant upstream fragment

3' ACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

000201" 09/673739" 102000

6/7

**A** Reverse primer SEQ ID NO:23  
 5' GCTGTAAACGACGGCCAGTTTCAT 3'  
 5' GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3'  
 Synthetic template No.1 SEQ ID NO:22  
 X X  
 3' CGACCACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 Normal upstream fragment  
 SEQ ID NO:20

PCR amplification in presence of  $\alpha^{32}\text{P}$ dCTP  
 followed by denaturing PAGE

SEQ ID NO:24  
 3' CGACATTTGCTGCCGGTCAAAGTACGTCCCGACACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 66mer

**B** Reverse primer SEQ ID NO:23  
 5' GCTGTAAACGACGGCCAGTTTCAT 3'  
 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3'  
 Synthetic template No.2 SEQ ID NO:25  
 XXXXX  
 3' CGACCACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 Normal upstream fragment  
 SEQ ID NO:20

PCR amplification in presence of  $\alpha^{32}\text{P}$ dCTP  
 followed by denaturing PAGE

X

**C** Reverse primer SEQ ID NO:23  
 5' GCTGTAAACGACGGCCAGTTTCAT 3'  
 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3'  
 Synthetic template No.2 SEQ ID NO:25  
 XXXXX  
 3' ACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 Mutant upstream fragment  
 SEQ ID NO:21

PCR amplification in presence of  $\alpha^{32}\text{P}$ dCTP  
 followed by denaturing PAGE

SEQ ID NO:26  
 3' CGACATTTGCTGCCGGTCAAAGTACGTCTAGGTACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 66mer

**D** Reverse primer SEQ ID NO:23  
 5' GCTGTAAACGACGGCCAGTTTCAT 3'  
 5' GCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3'  
 Synthetic template No.1 SEQ ID NO:22  
 X X  
 3' ACCGCATCCGTTCTCACGGAAGTCTATGTGCG 5'  
 Mutant upstream fragment  
 SEQ ID NO:21

PCR amplification in presence of  $\alpha^{32}\text{P}$ dCTP  
 followed by denaturing PAGE

X

Mismatches denoted by X  
 $^{32}\text{P}$  label denoted by \*

FIG 6

00673739.102000

7/7

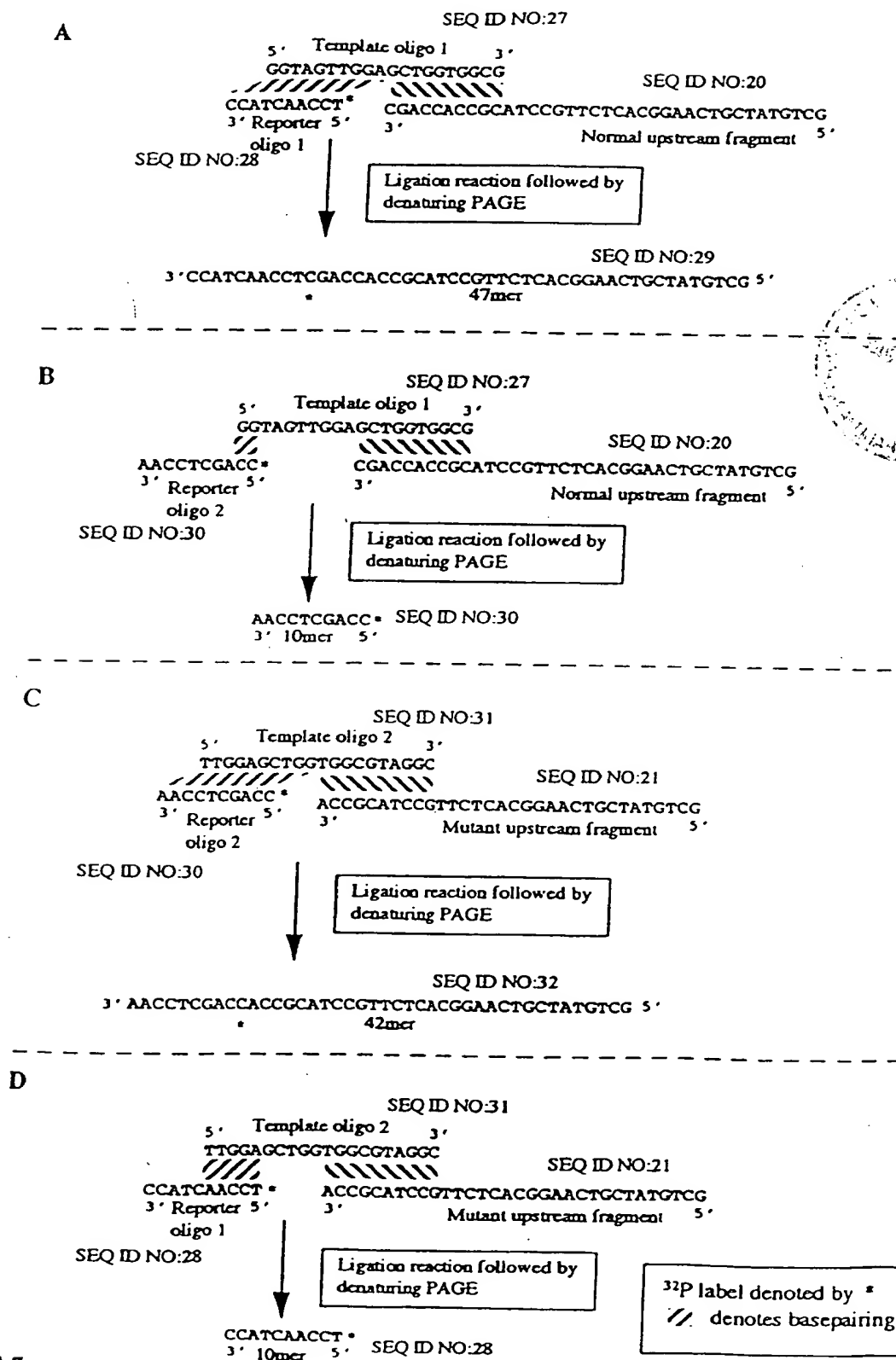


FIG 7

000201 6622960